Laboratory Manager Approval: Mary K. Bowman / 08/19/2021

QA Manager Approval: Jeffrey Moore / 08/19/2021

Fecal Coliform Membrane Filter (MF) Method Standard Method 9222D

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1. Scopes and Application

1.1 The membrane filter technique is one of the basic procedures used in the detection of coliform. The procedure involves filtering a certain volume of sample, plating the filter on a specific medium, and incubating at the desired temperature. The best readable plate is then counted for its colonies, the colonies verified, and the density calculated using the count and the volume of sample filtered. With the advent of the faster enzyme substrate method, the membrane filter technique is not used for testing everyday drinking water samples; however, it is performed primarily for a special study involving State Parks and Special Projects. Direct plating methods such as the membrane filter procedure permit a direct count of coliform colonies. The volumes tested are 100, 50 and 10 mls, the media is M-FC Broth, and the samples are incubated at a temperature of 44.5 ±0.2 degrees Celsius in a water bath. The best readable plate is one that contains 20-60 BLUE colonies per plate. The best selection would be the 100 ml plate or a plate closest to 100 mls.

2. Definitions

2.1 Refer to Chapter 3 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions.

3. Interferences

3.1 Method interferences may be caused by contaminants in reagents, media, bottles, glassware, filters, and other processing apparatus. To abstain from interferences, all reagents and media are tested for sterility prior to use. Also, all bottles and glassware are sterilized and tested prior to usage.

3.1.1 Each batch of media is aseptically prepared according to manufacturers instructions, sterilized, and tested before being used. The media in this procedure is EC broth. The commercially prepared media used in this is M-FC Broth. A negative and positive control is used each week to ensure its quality assurance.

3.1.2 Dilution water is aseptically prepared and sterilized according to prescribed methods. Each batch of water is tested for sterility.

3.1.3 All glassware must be washed, sterilized, and put in the hot air oven at 180°C for 2 hours. A pH check is performed on all batches of glassware using a 0.04% solution of bromothymol blue. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants.

3.1.4 All bottles are washed and sterilized prior to use. Sodium Thiosulfate is added to the round collection bottles to absorb any excess chlorine residual that may come from the water sample.

4. Safety

4.1 Refer to Laboratory Chemical Hygiene Plan, online revision.

5. Apparatus and Equipment

5.1 Round - 250 ml (8 oz) and 125 ml (6 oz) Nalgene bottles.
5.2 44.5 ± 0.2 °C Water Bath
5.3 UV Light Boxes (Black Boxes)
5.4 Dissecting Microscope
5.5 Colony Counter
5.6 Vacuum Manifold
5.7 Smooth-tipped Forceps
5.8 Bunsen Burner
5.9 Sterile 10 ml Pipets
5.10 Sterile Cellulose Acetate Filters (Millipore - 0.45 um, white grid, 47 mm)
5.11 Petri Dishes with pad (50x11 mm)
5.12 Sterile Wooden Applicator Sticks (6" x 1/12")
5.13 50 ml graduated cylinders

6. Reagents

6.1 M-FC Broth
6.2 EC Broth
6.3 Ethanol
6.4 Dilution/Rinse Water

7. Sample Collection

7.1 Refer to Chapter 5 of the Georgia EPD Laboratory Quality Assurance Manual for Sample Container, Sample Preservation and Sample Holding Times.

8. Calibration

8.1 There are no calibrations associated with this method. Maintain sterility with equipment media and technique. Maintain the water bath at the proper temperature 44.5 ± 0.2 degrees Celsius.

9. Quality Control

9.1 Refer to Table 13.1 Quality Control Acceptance Criteria associated with this method.

10. Procedure

10.1 Set up station, using sterilized equipment and filters. Check UV Black Box to ensure UV light is functioning properly.

10.2 Turn vacuum on. Place bottom half of suction funnel on manifold. (Make sure manifold vacuum port is in the close position.)

10.3 Take forceps out of alcohol (Ethanol) and flame. Place grid filter on funnels (grid side up). Then put top half of suction funnel (graduated and magnetized) on funnel bases with graduations facing you.

10.4 Run 100 ml of dilution water first and last as controls. (Be careful to always flame dilution water nozzle prior to using.)

10.5 Shake sample vigorously about 25 times. In the first funnel, pour in 100 ml of sample. In the second funnel pour in 50 ml of sample. (Use the graduated cylinder to measure your sample.) Next, use the pipette to put 10 ml of sample in your third funnel.

10.6 Turn on the manifold vacuum opening port to draw the sample through the filter. Immediately turn off the vacuum part of each funnel once all of the water has been filtered.
Note: A turbid sample will take longer to filter then a clean sample.

10.7 Rinse sides of funnel using dilution water, approximately 30 to 50 mls.

10.8 After all water has been drawn through the filter, turn off vacuum port and remove funnels, placing them in the Black Box.

10.8.1 Immediately turn UV lights on in the Black Box to sterilize funnels for the next set of samples. Decontaminate by exposing to ultraviolet radiation for two minutes.

10.9 With an indelible pencil, label all filters with sample number and proper dilution. (Likewise, label all controls.)

10.10 Roll filters onto plates with sterile forceps (dipping forceps in EtOH and flaming between each sample). This rolling motion avoids entrapment of air under the filter.

10.11 Repeat steps 10.3 to 10.10 until all samples are analyzed. Make sure to keep all samples in order.

10.12 Run 100 ml of an EC sample as a positive control, following the same procedure.

10.13 Put plates upside down in a plastic bag. Make sure bag is air tight so that no water will seep in. Label bag appropriately and place in water bath at 44.5 ± 0.2°C for 24 ± 2 hours. Note: It is best to place bag through a test tube rack and weigh down (to prevent bag from floating to the top). All plates should be placed in water bath within 30 minutes after filtration.

11. Calculations

11.1 Since each sample has a choice of three plates, select the one that has 20-60 colonies. If it is the 100 volume sample, then record the number of colonies read. If the 50 or 10 ml plate is chosen, multiply the number of colonies read by 2 or 10, respectively, to obtain the density. If none of the plates are ideal (below the range), then use the following equation.

$$\text{Density} = \frac{\text{The total number of colonies from all three plates}}{\text{The total volumes used}} \times 100$$

12. References

12.1 Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association: Washington, DC, 1998.

13. Practice Quantitation Limits (PQLs), Precision and Accuracy Criteria and Quality Control Approach

Table 13.1 Summary of Data Quality Objectives

Method	Parameter	QC Check	Min. Frequency	Accepted Criteria	Corrective Action	Flagging Criteria
SM 9222D - Fecal Coliform Membrane Filter (MF) Technique	Positive Control	Known EC plated	1 each run	Production of Blue Colonies	Order new lot of media	Positive for E. coli
	Negative Control	Sterile dilution water filtered on plate.	1 per jug of dilution water.	No Growth on plate	Discard the controls and the run. Reanalyze all	Negative
	Number of Colonies on Plate		Each	20-60 colonies per plate	Find plate that falls within range.	Count colonies and Calculate
	Colony Inspection		Each	Blue Colonies	Colonies are recorded as atypical.	Accurate colonies selected
	Blue Colonies	Verification in EC Media	10 per plate	Gas production	None	Positive for Fecal Coliforms